Gonadal steroid synthesis in the Virginian opossum, *Didelphis marsupialis*. By BRIAN COOK,* NATALIE S. SUTTERLIN, JEAN W. GRAUER and A. V. NALBANDOV. *Department of Animal Science, Animal Genetics Laboratory, University of Illinois at Urbana-Champaign, U.S.A., and *Department of Steroid Biochemistry, University of Glasgow, Royal Infirmary, Glasgow, G4 0SF*

Previously, we have shown that, although opossum ovaries 6 days after luteinization synthesized progesterone *in vitro*, and converted [^{3}H]pregnenolone and [^{3}H]cholesterol into [^{3}H]progesterone, they did not incorporate [^{14}C]acetate into progesterone (Cook & Nalbandov, 1968). Because morphological regression of the corpus luteum of the opossum begins on day 7 (Hartman, 1923), the possibility was examined that lack of acetate incorporation was due to senescence. Ovaries were luteinized by injection of pregnant-mare serum gonadotrophin followed 72 h later by injection of human chorionic gonadotrophin (HCG). Pairs of animals were killed 0, 0·5, 1, 1·5, 2, 3, 4 and 6 days after HCG injection. Ovarian slices were incubated in one of four substrates: [^{14}C]acetate (with and without glucose), [^{3}H]cholesterol or [^{3}H]pregnenolone. At no time was [^{14}C]acetate incorporated into progesterone. At day 0, progesterone concentration in the tissue, after incubation, was about 300 ng/mg. At day 1·5 it dropped to 140 ng/mg, on day 4 it rose to 200 ng/mg and on day 6 it declined again to 140 ng/mg. [^{3}H]Progesterone production from [^{3}H]pregnenolone and [^{3}H]cholesterol followed this biphasic pattern as did the concentration of progesterone in peripheral plasma. We attribute initial high progesterone values to stimulation by exogenous gonadotrophin; as time from injection increased, stimulation decreased. Growth of luteal tissue produced the second peak on day 4, then regression followed. Since opossum ovaries did not incorporate acetate, testes were examined similarly.

Testicular slices incubated for 3 h incorporated appreciable quantities of [1-^{14}C]acetate into both androstenedione and testosterone. An increase in both [^{14}C]androstenedione ($P < 0·01$) and [^{14}C]testosterone ($P < 0·05$) production was observed when luteinizing hormone (LH) (2 μg/ml) was added to the incubation medium. A second experiment utilized a 2 × 2 factorial design and showed that priming animals with HCG for 3 days before incubation increased [^{14}C]androstenedione ($P < 0·02$) and [^{14}C]testosterone ($P < 0·01$) production. Addition of LH (2 μg/ml) *in vitro* also increased ($P < 0·05$) both [^{14}C]androstenedione and [^{14}C]testosterone production. The effect of LH *in vitro* was the same, regardless of whether animals had been primed. Neither HCG *in vivo* nor LH *in vitro* influenced the ratio of [^{14}C]testosterone to [^{14}C]androstenedione. The conversion of [^{3}H]cholesterol and [^{3}H]pregnenolone to [^{3}H]androstenedione and [^{3}H]testosterone by incubated testicular slices was also demonstrated. Androstenedione and testosterone were shown by gas chromatography to be present in male peripheral blood. This is probably the first confirmation that these steroids are produced in *Didelphis*.

We conclude that the ovary of the opossum does not incorporate acetate into steroids, whereas the testis does. Morris & Chaikoff (1959) and Gerson, Shortland & Dunckley (1964) suggested that cholesterol found in the testis is synthesized *in situ*, whereas Solod, Armstrong & Greep (1966) have shown that the ovary obtains cholesterol from circulating blood. The opossum provides unique, direct support for these ideas.

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Preparation of antisera to luteinizing hormone releasing hormone. By H. M. FRASER, A. GUNN, S. L. JEFFCOATE* and DIANE T. HOLLAND.* *Department of Surgery, University of Dundee, Dundee, DD1 4HN and *Department of Chemical Pathology, St Thomas's Hospital, London, S.E.1*

For the purpose of raising antibodies to luteinizing hormone releasing hormone (LH-RH) we have conjugated the decapeptide to bovine serum albumin (BSA) in order to increase immunogenicity. This has been accomplished using bis-diazotized benzidene (BDB) and carbodiimide.

Journal of Endocrinology v·61 1974

Luteinizing hormone releasing hormone was conjugated to BSA using BDB similar to the method described by Bassiri & Utiger (1972) for thyrotrophin releasing hormone (TRH). Conjugation by carbodiimide was carried out by reacting together 3 mg LH-RH, 3 mg BSA and 10 mg 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide in 0·75 ml water overnight at room temperature and dialysing against distilled water for 48 h and against 0·15 M-NaCl for 24 h. Immunization was by emulsification in Freund's complete adjuvant and injection at multiple intradermal sites in two animals followed by 0·5 ml pertussis vaccine.

Conjugation by BDB takes place through the histidine and/or tyrosine residues of LH-RH. The most likely site of conjugation by carbodiimide is through the hydroxyl group of tyrosine. This conclusion is supported by cross-reaction studies (Jeffcoate, Holland, Fraser & Gunn, 1974) which demonstrate the greatest antigenicity at the CO_2H-terminal end of the molecule. This method of conjugation has been employed most frequently, all eight rabbits and six rats immunized with the conjugate produced antibody which appeared within 6 weeks. Five of the rabbit antisera have been used for radioimmunoassay of LH-RH, being of high avidity and specificity. The rabbit antisera previously described (Jeffcoate, Fraser, Gunn & Holland, 1973) has an affinity constant (K), calculated according to the method of Scatchard (1949), of 7×10^9 l/mol.

The relative unimportance of the first two NH_2-terminal amino acids immunologically was demonstrated by the 3–10 octapeptide showing complete cross-reactivity. Consequently, this indicated the possibility of raising antisera to the octapeptide which would cross-react with LH-RH. The availability of the α-amino group on the octapeptide allows it to be readily conjugated to BSA by carbodiimide. Fourteen rats have been used to raise antisera to the 3–10 octapeptide. All have produced antibody which cross-reacts with LH-RH *in vitro*. This cross-reactivity was confirmed by our finding that similar in-vivo effects occur in rats as described for rabbits immunized with LH-RH (Fraser & Gunn, 1973*a*, *b*). Specificity studies with these antisera are at present being carried out. One of these antisera has been used to develop a highly sensitive assay for LH-RH. The usable range of the assay is from 0·1 to 30 pg LH-RH/tube and the affinity content for this antiserum is $1·6 \times 10^{11}$ l/mol.

We have demonstrated that antisera can be readily generated to LH-RH by conjugation to BSA. These antisera are highly specific and suitable for radioimmunoassay of LH-RH allowing it to be detected in biological fluids. The fragment which would show cross-reaction in biological fluids is the 3–10 octapeptide which may occur as a metabolite. These antisera are also being used to study the action of LH-RH by neutralization *in vivo*. Our findings also suggest that the rat may be a useful species for raising antisera for radioimmunoassay.

We are grateful to Dr W. Bogie (Hoechst Pharmaceuticals Ltd) for supplies of synthetic LH-RH and the octapeptide.

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Effects of antiserum to luteinizing hormone releasing hormone during the oestrous cycle of the rat. By H. M. Fraser and A. Gunn. *Department of Surgery, University of Dundee, Dundee, DD1 4HN*

We have demonstrated that antibody to luteinizing hormone releasing hormone (LH-RH) is effective in neutralizing LH-RH in immunized rabbits and when passively transferred in the rat (Fraser & Gunn, 1973*a*, *b*). The antiserum (Jeffcoate, Holland, Fraser & Gunn, 1974) has been utilized to investigate the role of LH-RH during the rat oestrous cycle by neutralizing LH-RH at various times.

Animals studied were normal female Sprague-Dawley rats maintained under controlled lighting (lights on at 05.00 h and off at 19.00 h). Only rats showing regular 4-day cycles were used. Under these conditions plasma luteinizing hormone (LH) reaches highest values (over 1000 ng NIAMD-LH-RP1/ml) between 17.00 and 18.00 h on the afternoon of pro-oestrus. A group of animals were given tail vein injections of 1·0 ml antiserum, under light ether anaes-

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